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Enzyme Catalyzed Intramolecular Cannizzaro Reaction of Phenylglyoxal

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ABSTRACT

Novozyme 435 (commercially immobilized lipase B from *Candida antarctica*) was found to catalyze the intramolecular Cannizzaro reaction of phenylglyoxal in aqueous medium forming mandelic acid. Simultaneously, the oxidation of phenylglyoxal to phenylglyoxylic acid was also found to take place. For optimization of conversion of each of these products, different enzymes were tried as the biocatalyst. The presence of various organic co-solvents was also found to have a marked effect of the course of the reaction.

KEYWORDS

Intramolecular Cannizzaro reaction, Novozyme 435, Aqueous medium, Organic co-solvents, Catalytic promiscuity.

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INTRODUCTION

Enzymes form an important part of the marvellous tool box of catalysts that nature offers to mankind. Over the years, organic chemists have been expanding their knowledge about how to utilize this set of catalysts for the synthesis of important molecules. While deciding the synthetic strategy for a particular compound, the enzymatic route is often found to be 'greener' and more 'sustainable' than most of the other available alternatives [1,2]. In recent years, catalytic promiscuity of enzymes has further increased the scope of biocatalytic reactions [3-6].

Mandelic acid and its derivatives act as important building blocks for the synthesis of pharmaceuticals and fine chemicals [7]. Intramolecular Cannizzaro reaction carried out using α-keto aldehydes such as phenylglyoxal is a useful way of synthesizing these compounds. Various chemical catalysts have been used for catalyzing the intramolecular Cannizzaro reaction of phenylglyoxal which forms mandelic acid as the product. Traditionally, strong bases such as NaOH have been used for this purpose [8,9]. The proposed mechanism for the alkali catalyzed reaction involves an intramolecular hydride transfer [8,10]. Hine & Fischer have described the internal catalysis in the reaction of N,N,N′-trimethylethylenediamine with phenylglyoxal to the give the corresponding mandelamide derivative [11]. Cobalt-Schiff base complexes have been used for catalyzing highly selective conversion of aryglyoxals to the corresponding α-aryl-α-hydroxyacetic esters in alcohols as the reaction medium [12]. The authors proposed that the Lewis acidity of Co(III) may be reason behind this reaction.

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When this reaction of phenylglyoxal catalyzed by the cobalt complexes was carried out in the presence of oxygen, it also yielded the corresponding phenylglyoxylate as a minor product. Russel and co-workers carried out the asymmetric version of this reaction using Cr(III) and perchlorate salts [13]. In recent years, there have been several other reports about the use of Lewis acids as efficient catalysts for this reaction [14-16]. Wang *et al.* used amorphous, non-porous silica/alumina prepared by flame spray pyrolysis for conversion of phenylglyoxal to alkyl mandelates [17]. Although the yield and/or enantioselectivity of the product is reported to be reasonably high in most of these cases, yet the reaction conditions employed require either high temperatures or toxic organic solvents. Thus there is an impending need of newer, environmentally benign ways of catalyzing such reactions.

Among biocatalysts, the glyoxalase enzyme system is known to catalyze the conversion of methylglyoxal and phenylglyoxal to lactic acid and mandelic acid respectively [18,19]. Recently, we had shown that lipases especially, Novozyme 435 can catalyze the Cannizzaro-type reaction of substituted benzaldehydes in water [20]. Herein, we report the ability of a lipase to catalyze the intramolecular version of this reaction under simple conditions. Using phenylglyoxal hydrate as a model substrate, aqueous buffer as the reaction medium, Novozyme 435 as the catalyst and at ambient temperature, mandelic acid was obtained. A simultaneous oxidation reaction also occurred forming phenylglyoxylic acid (**Scheme-I**).

EXPERIMENTAL

Novozyme 435 (*Candida antarctica* lipase B immobilized on polyacrylic resin), Lipozyme CALB, Lipozyme RMIM (*Rhizomucor miehei* lipase immobilized on anion exchange resin) were kind gifts from Novozymes, Bagsvaerd, Denmark. Lipase M (*Mucor javanicus* lipase) was a kind gift from Amano Enzymes Inc., Nagoya, Japan. Acetonitrile (99.9 %, HPLC grade), DMSO (99.8 %, anhydrous grade) were obtained from Sigma, St. Louis, USA. Phenylglyoxal hydrate and D, L-mandelic acid were obtained from Merck, Germany. Phenylglyoxylic acid (benzoyl formic acid) was procured from Spectrochem, Mumbai, India. All other chemicals were of analytical grade and were used without further purification.

Novozyme 435 catalyzed reaction of phenylglyoxal: A solution of phenylglyoxal (1 mM) in 100 mM sodium phosphate buffer, pH 7.0, was shaken with 20 mg lipase at 30 °C and 200 rpm. Total reaction volume was 1.25 mL. Aliquots of 40 µL were taken at different points of time.

HPLC analysis: Each aliquot was diluted with 600 µL acetonitrile to precipitate the enzyme. This precipitated enzyme was removed by centrifugation. The supernatant was analyzed by HPLC using a Zorbax C-18 reverse phase column. The eluent was a mixture of water/acetonitrile/acetic acid (95/5/0.2) at a flow rate of 1.1 mL/min. Analysis was carried out at two wavelengths: 225 nm and 254 nm by a procedure similar to that described by Ogata & Sugihara [21]. Retention times of the peaks were matched with those of commercially available compounds; phenylglyoxal (t_R = 18.6 min), phenylglyoxylic acid (t_R = 6 min), mandelic acid (t_R = 11.4 min).

Product extraction and purification: The reaction of phenylglyoxal hydrate catalyzed by Novozyme 435, scaled up to a volume of 360 mL, was carried out as described earlier. After a period of 72 h, the enzyme was separated by filtration. The aqueous extract was concentrated, followed by extraction with dichloromethane. The organic layer was dried with anhydrous sodium sulphate and concentrated by rotary evaporation. The crude product was purified by column chromatography to yield mandelic acid and phenylglyoxylic acid.

Characterization of the reaction products: Both phenylglyoxylic acid and mandelic acid were characterized by ^{1}H NMR (Bruker-S-300) spectroscopy. The spectra obtained matched with those of the commercially available compounds.

RESULTS AND DISCUSSION

Lipase catalyzed reactions of phenylglyoxal hydrate in aqueous medium: To begin with, Novozyme 435 (which had proved to be the ideal enzyme in our work with substituted benzaldehydes) was chosen as the biocatalyst for this reaction. Fig. 1 shows the time course for this reaction carried out in 100 mM sodium phosphate buffer, pH 7.0 as the reaction medium.

Analysis of the reaction by HPLC showed that initially, the oxidation of phenyl glyoxal to phenyl glyoxylic acid (or

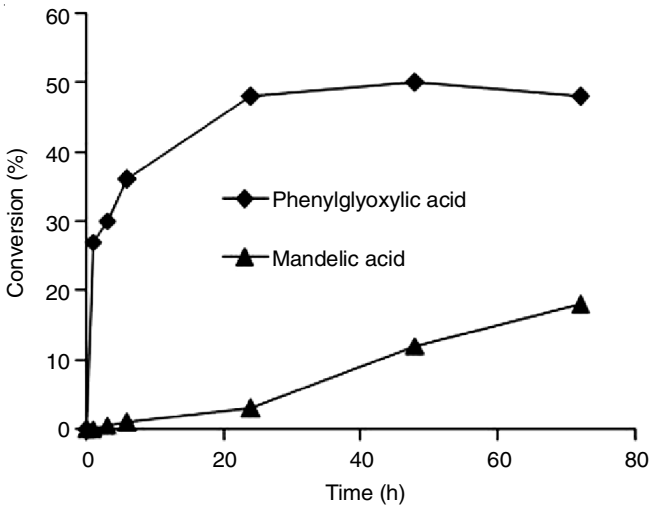
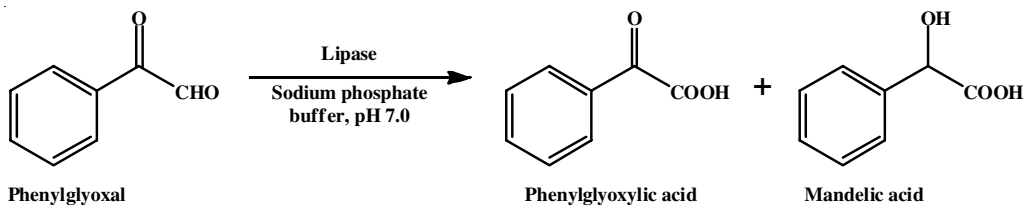


Fig. 1. Time course for the Novozyme 435 catalyzed reaction of phenylglyoxal in water



Scheme-I: Lipase catalyzed reaction of phenylglyoxal in aqueous medium giving phenylglyoxylic acid and mandelic acid

benzoyl formic acid) was the major reaction; with almost 50 % of the substrate getting oxidized after 24 h. Till this time, only about 4 % conversion to mandelic acid had taken place. Thereafter, the amount of phenyl glyoxylic acid formed did not increase any further. However, the conversion to mandelic acid increased. After 72 h, about 20 % mandelic acid conversion was obtained. The control reaction run in the absence of enzyme did not lead to any significant conversion after 96 h.

Biocatalyst screening: A screening of some of the commercially available lipases was then carried out. Table-1 shows the product composition obtained after a period of 24 h. It can be seen that while CALB (both free as well as immobilized) and *Mucor miehei* lipase (immobilized) gave both the products *i.e.* mandelic acid and phenylglyoxylic acid, in case of *Mucor javanicus* lipase no products could be detected even after 24 h. Also the urea denatured Novozyme 435 led to only 4 % phenylglyoxylic acid, while no mandelic acid was formed. A control run in the absence of any enzyme also did not lead to any significant conversion, indicating the specific role played by the lipase during the reaction.

Effect of the reaction medium: Medium engineering is an established approach for use of enzymes for organic synthesis [22]. Presence of organic solvents in the reaction medium is known to have marked effects on the course of many enzyme catalyzed promiscuous reactions as well [23-25]. In case of the lipase catalyzed Cannizzaro-type reaction of *p*-nitrobenzaldehyde, presence of organic co-solvents was found to almost completely inhibit the oxidation of the aldehyde to the acid [20]. Thus, it was decided to examine the effect of addition of organic co-solvents on the Cannizzaro reaction of phenylglyoxal as well. During our initial investigation, DMF was added as the co-solvent to the reaction medium. The amount of DMF added was varied from 0 to 15 % (v/v) with respect to total reaction volume. Table-2 shows the effect of varying the concentration of DMF on the product composition. Clearly, increasing the amount of DMF from 0 to 10 % led to better conversions for both mandelic acid as well as phenylglyoxylic acid. However, a further increase in its amount did not improve the conversions any more. The reaction medium containing 10 % DMF proved to be the most suitable in terms of the conversions of both phenylglyoxylic acid and mandelic acid.Thus, for further work the amount of organic co-solvent added was fixed at 10 % (v/v).

As a next step, two more organic solvents were chosen, one having lesser hydrophobicity than DMF and the other one being more hydrophobic. These were DMSO (log P = -1.35) and dioxane (log P = -0.27). Figs. 2-4 show the time course for the reactions carried out in the presence of 10 % (v/v) DMF, DMSO and dioxane respectively.

It can be seen that the presence of all the solvents selected in the present study had varying effects on formation of the two products. A comparison of the product composition after 24 h shows that in presence of DMF, 50 % phenylglyoxylic

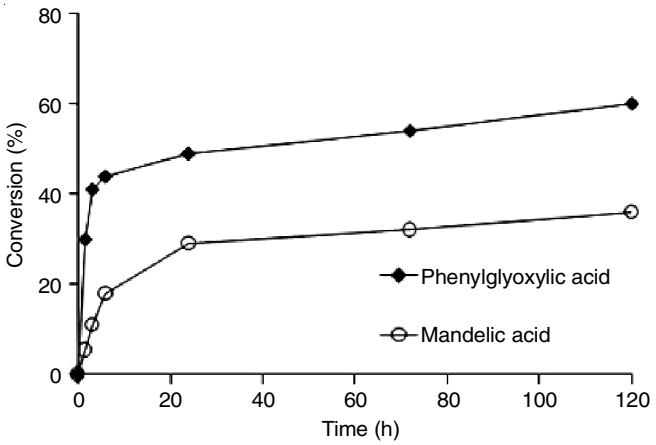


Fig. 2. Effect of DMF (10 % v/v) on the Novozyme 435 catalyzed reactions of phenylglyoxal

TABLE-1
SCREENING OF A FEW LIPASES FOR THE REACTION OF PHENYLGLYOXAL IN WATER[a]

Entry	Biocatalyst	Phenyl glyoxylic acid (%)[b]	Mandelic acid (%)[b]
1	Novozyme 435[c]	54	3
2	Lipozyme CALB[d]	51	11
3	RMIM[e]	47	4
4	Lipase M[f]	0	0
5	Urea denatured Novozyme 435[g]	4	0
6	No enzyme	0	0

[a]Reaction conditions: A solution of phenylglyoxal hydrate (1 mM) in 100 mM sodium phosphate buffer was shaken with 20 mg biocatalyst at 30 °C, 200 rpm; [b]Corresponds to conversion values obtained by HPLC analysis after a period of 24 h; [c]Novozyme 435 is commercially immobilized *Candida antarctica* lipase B; [d]Lipozyme CALB is free *Candida antarctica* lipase B; [e]RMIM is commercially immobilized *Rhizomucor miehei* lipase; [f]Lipase M is free *Mucor javanicus* lipase; [g]Enzyme was denatured by incubating overnight with 8M urea at 100 °C.

TABLE-2
EFFECT OF ADDITION OF VARYING AMOUNTS OF DMF ON THE NOVOZYME 435 CATALYZED REACTIONS OF PHENYLGLYOXAL[a]

Time (h)	0 % DMF (v/v)		5 % DMF (v/v)		10 % DMF (v/v)		15 % DMF (v/v)	
	PGA (%)	MA (%)	PGA (%)	MA (%)	PGA (%)	MA (%)	PGA (%)	MA (%)
1	27	0.5	28	3	30	5	27	2
3	30	1	33	9	41	11	31	6
24	48	3	49	24	49	29	44	18
72	48	18	49	26	54	32	50	21

[a]Reaction conditions: A solution of phenylglyoxal hydrate (1 mM) in 100 mM sodium phosphate buffer containing varying amounts of DMF was shaken with 20 mg Novozyme 435 at 30 °C, 200 rpm. PGA is phenylglyoxylic acid and MA is mandelic acid.

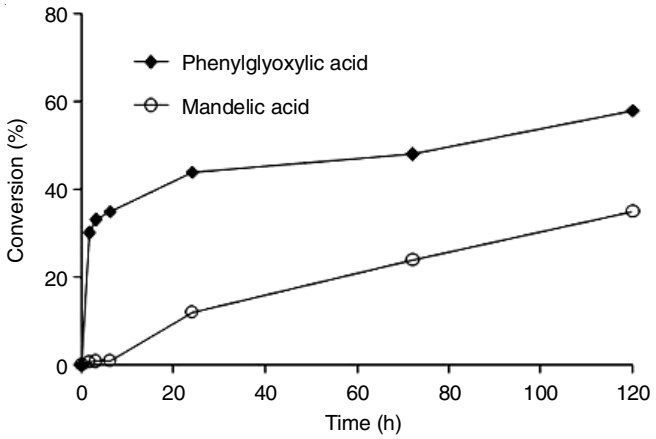


Fig. 3. Effect of DMSO (10 % v/v) on the Novozyme 435 catalyzed reactions of phenylglyoxal

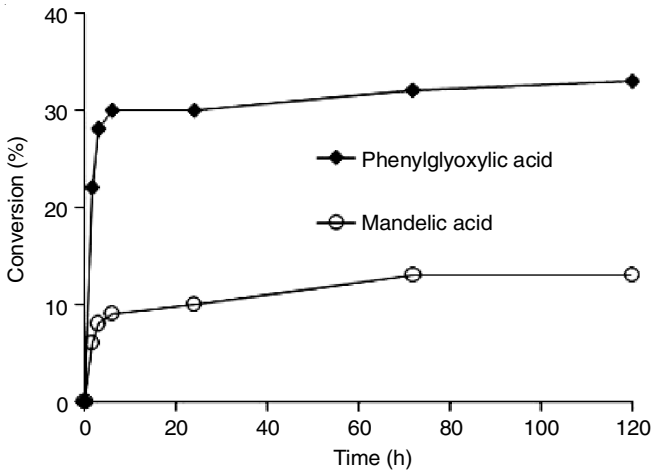


Fig. 4. Effect of dioxane (10 % v/v) on the Novozyme 435 catalyzed reactions of phenylglyoxal

acid and 30 % mandelic acid were obtained; 40 % phenylglyoxylic acid and 10 % mandelic acid in case of DMSO; 30 % phenylglyoxylic acid and 10 % mandelic acid were obtained in case of dioxane. Thus, in terms of overall conversion as well % conversion of mandelic acid, DMF performed better than the other two solvents. Interestingly, in all these cases it was the oxidation product *i.e.* phenylglyoxylic acid that was formed faster than mandelic acid and also continued to be the major product. This observation is in contrast with that made in the case of Novozyme 435 catalyzed Cannizzaro-type reaction of *p*-nitrobenzaldehyde [20]. In case of the *p*-nitrobenzaldehyde, addition of organic solvents to the reaction medium had resulted in almost complete inhibition of oxidation of the aldehyde to the acid.

Apart from the well-established, classical intramolecular hydride transfer mechanism for Cannizzaro reaction of arylglyoxals catalyzed by chemical catalysts [8,10], the glyoxalase enzyme system is known to catalyze this reaction *via* an endiol proton transfer mechanism [18,19]. The detailed studies about the mechanistic pathways of many lipase catalyzed promiscuous reactions are still going on [26,27]. We had reported earlier that the Cannizzaro-type reaction of substituted benzaldehydes does not follow the hydride transfer mechanism alone and that there is a possible involvement of radical-anion species in that case [20]. An investigation about the mechanistic aspects of the reaction involving lipase and phenylglyoxal could possibly explain the results observed by us. However, the following important conclusions can be drawn on the basis of the work carried out so far: (a) lipases especially *Candida antarctica* lipase B can catalyze the intramolecular Cannizzaro reaction as well as oxidation of phenyl glyoxal in aqueous medium. (b) It was possible to change the extent of these two reactions by addition of organic co-solvents.

Conclusion

It has been shown that lipases are capable of catalyzing intramolecular Cannizzaro reaction and that too under environmentally benign conditions: aqueous buffer with or without small amount of organic co-solvent was used as the reaction medium, no external redox reagent was required to bring about the reactions. This enzyme catalyzed method of synthesizing mandelic acid and phenylglyoxylic acid provides a useful 'green' alternative to the existing synthetic methodologies.

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